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PHYTOCHEMICAL SCREENING, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY FROM *SONNERATIA CASEOLARIS* LEAVES EXTRACT

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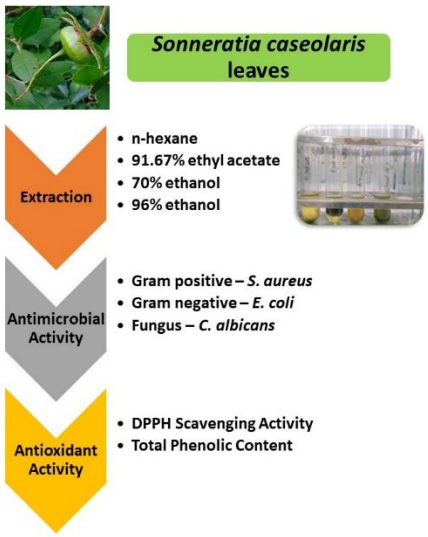
Graphical abstract



Abstract

This work deals with the screening of phytochemical compounds, and the analysis of the antimicrobial and antioxidant activity of *Sonneratia caseolaris* leaves from Maros Regency, South Sulawesi Province, Indonesia. The phytochemical compounds were extracted separately using n-hexane, ethyl acetate, 70% and 96% ethanol. The antimicrobial activity was assessed by measuring the clear zone in the agar diffusion method. The phytochemical screening detected the presence of alkaloids, tannins, saponins, phenolics and flavonoids extracted by four different solvents, n-hexane, ethyl acetate, 70% ethanol and 96% ethanol. The ANOVA results showed that n-hexane and 70% ethanol extract had significant antimicrobial activity ($P<0.05$) against *E. coli*, *S. aureus* and *C. albicans,* while ethyl acetate extract had no significant ($P>0.05$) antimicrobial activity against these three microbes. The antioxidant activity was determined using the DPPH radical scavenging assay, and the total phenolic content was determined using the Folin-Ciocalteu method. The highest radical scavenging activity of 80.21% was found in 96% ethanol extract, and the lowest antioxidant activity of 16.71% was found in ethyl acetate extract. The total phenolic content is expressed as mg of gallic acid equivalent (GAE) per gram of the extract, with the highest phenolic content, 74.77 mg GAE/g, found in the 70% ethanol extract. Meanwhile, n-hexane extract had the lowest total phenolic content of 4.67 mg GAE/g. These findings showed that *S. caseolaris* leaves extracts have antimicrobial and antioxidant activities, thus suggesting their potential as natural antimicrobials or antioxidants in the medicinal and food processing industries.

Keywords: Phytochemical, antimicrobial activity, antioxidant activity, *Sonneratia caseolaris*, mangrove plants

Abstrak

Kajian ini berkenaan penyaringan fitokimia dan aktiviti antimikrobial dan antioksidan daun *Sonneratia caseolaris* dari Kabupaten Maros, Sulawesi Selatan, Indonesia. Sebatian fitokimia diekstrak secara berasingan dengan empat pelarut: n-heksana, etil asetat, 70% dan 96% etanol. Aktiviti antimikrobial dinilai dengan mengukur diameter zon jernih yang diperoleh dari kaedah serapan agar. Sementara itu, penyaringan fitokimia mendapati ekstrak daun *S. caseolaris* mengandungi sebatian alkaloid, tanin, saponin, fenolik dan flavonoid. Analisis ANOVA mendapati, ekstrak daun dari pelarut 70% etanol mempunyai aktiviti antimikrobial yang signifikan ($P<0.05$) terhadap pertumbuhan *E.coli*, *S. aureus* dan *C. albicans*, manakala ekstrak daun dari etil asetat tidak mempunyai aktiviti antimikrobial yang signifikan ($P>0.05$) terhadap ketiga-tiga mikrob yang diuji. Aktiviti antioksidan diuji dengan menggunakan kaedah pengorekan aktiviti radikal DPPH, dan jumlah kandungan fenolik ditentukan dengan menggunakan kaedah Folin-Ciocalteu. Aktiviti pengorekan radikal bebas tertinggi dicatatkan oleh ekstrak daun dari pelarut 96% etanol iaitu sebanyak 80.21%, dan aktiviti pengorekan radikal bebas terendah dicatatkan oleh ekstrak daun dari pelarut etil asetat. Jumlah kandungan fenolik dicatatkan dalam unit mg of gallic acid equivalent (GAE) per g ekstrak, dengan kandungan tertinggi sebanyak 74.77 mg GAE/g ekstrak dicatatkan oleh ekstrak dari pelarut 70% etanol, manakala ekstrak dari pelarut n-heksana mengandungi jumlah kandungan fenolik terendah iaitu sebanyak 4.67 mg GAE/g ekstrak. Dapatan ini menunjukkan ekstrak daun *S. caseolaris* mempunyai aktiviti antimikrobial dan antioksidan yang signifikan, dan ini mewajarkan potensi ekstak daun *S. caseolaris* sebagai sumber antimikrobial dan antioksida semulajadi untuk kegunaan perubatan dan industri pemprosesan makanan.

Kata kunci: Fitokimia, aktiviti antimikrobial, aktiviti antioksida, *Sonneratia caseolaris*, pokok bakau

1.0 INTRODUCTION

For centuries, aloe vera, *Amaranthus spinosus, Cascara bark, Stenochlaena palustris, Mangifera casturi, Eurycoma longifolia* Jack, *Sonneratia caseolaris* and many more have been long used as traditional medicine [1, 2]. Although modern medicine has made progress with high efficiency, some people still prefer the old way, traditional medicinal treatment, as its harmful effect is lesser [3]. Screening and isolating secondary metabolites from these traditional medicinal plants revealed compounds with antimicrobial activities such as alkaloids or antioxidant properties such as phenolic and flavonoids [4].

In the 20th century, antimicrobial agents were arguably the most successful drugs used. They were indispensable in many medical treatments such as intensive care, chemotherapy, organ transplants, premature baby care, and surgical procedures, which could not be carried out effectively without the availability of effective antibiotics [5, 6]. However, antibiotics in therapy are limited because bacteria have developed resistance to certain antibiotics, whether intrinsic resistance or acquired resistance. Bacteria produce resistance to exclude drugs from targets by decreasing cell wall permeability or drug destruction, reducing agent-altering enzymes, increasing the concentration of metabolites that antagonise drug action and forming adaptive drug inactivation enzymes [7].

Besides that, medicinal plants also have been a source of economic value in many parts of the world, especially its antimicrobials derivatives in traditional medicine [8]. For example, a methanolic extract of *S. caseolaris* fruits exhibited a high antimicrobial activity sensitivity against *C. albicans* and *S. aureus* [9]. In another study, an ethyl acetate and carbon tetrachloride extract of *S. caseolaris* stem were found to have the highest inhibitory effect against bacterial salmonella strains [10].

Besides antimicrobial, antioxidants are a particular group of nutrients produced by cells that eliminate free radicals [11]. In general, free radicals damage the functioning of glutathione peroxidase and regulate the work of the immune system, which leads to various diseases conditions [12, 13]. However, to consume antioxidants, it must have two conditions to be considered safe, which are the medium lethal dose (LD50) should not be less than 1,000 mg/kg body weight, and the antioxidant must not have a significant effect on the growth of experimental animals in long-term studies at a certain level [14].

In South Sulawesi, Indonesia, a mangrove plant (*Sonneratia caseolaris*) has been commonly applied for wound treatment. *S. caseolaris,* with a medium-size height of around 2 to 20 m and elliptic-oblong leaves

(5-9.5 cm long) [15] which is from the *Sonneratiaceae* family, can be easily found in coastal and estuary areas where other plants are difficult to grow [16]. Other than in wound treatment, *S. caseolaris* has been used for sprain, swelling, helminthiasis, poultices, coughs, hematuria, smallpox, astringent, antiseptic, arresting haemorrhage, piles, and stopping bleeding [17]. Studies on different parts of *S. caseolaris* such as stems, twigs, barks, leaves, and fruit extract had revealed secondary metabolites such as flavonoids, phenolics, terpenoids, steroids, alkaloids, saponin, tannin, and phenolic [18, 19, 20, 21, 15], which some of it responsible for its usage in wound treatment.

Even though *S. caseolaris* has been extensively used in wound healing, its specific medicinal compounds have not yet been described scientifically. A report by Pagarra *et al*. [20] focused on the *S. caseolaris* fruit from Maros Regency, South Sulawesi Province, which confirmed the presence of flavonoids, saponin, tannin, alkaloid, and phenolic. The antioxidant activity of *S. caseolaris* fruit was quite appreciable, and its antimicrobial activity was significant against *E. coli* and *C. albicans* but not significant against *S. aureus*. Therefore, this study focused on the chemical compound and bioactivity of *S. caseolaris* leaves harvested from the same province.

The objective of this study was to carry out a phytochemical screening and determination of the antioxidant and antimicrobial activities of *S. caseolaris* leaves. To the best of our knowledge, no previous work on phytochemical screening and bioactivity study has been published on *S. caseolaris* leaves from Maros, Regency, South Sulawesi Province, to evaluate its potential in medicinal treatment.

2.0 METHODOLOGY

Preparation of Plant Materials

S. caseolaris leaves were collected from the Maros Regency, South Sulawesi Province. The leaves were oven-dried and ground into powder for the extraction process.

Extraction Procedure

500 g *S. caseolaris* leaves powder was sieved and extracted by maceration using four solvents, 91.67% ethyl acetate, 70% ethanol, 96% ethanol and n-hexane for 24-hours at approximately 5000 mL (an accumulation of three times extraction). The filtrate obtained was evaporated using a rotary evaporator. Further, the extract was dried in an oven at 40 ℃ until a thick extract was obtained. The extract yield (in w/w%) was calculated using Equation 1.

$$\text{The yield} = \frac{(\text{Weight of extract})}{(\text{Dry weight of the sample})} \times 100\% \quad (1)$$

Phytochemical Test

of the content of these active compounds was carried out in several ways, namely the alkaloid test, flavonoid test, tannin test, saponin test, and phenolic test [22].

Antibacterial Activity

According to the Murray *et al*. [23] method, antibacterial activity was carried out with slight modification. The bacteria used were *Staphylococcus aureus* and *Escherichia coli*. To test the antibacterial activity of *S. caseolaris* leaves extract, 100 µL of bacterial spectrums (108 CFU/mL bacteria) were spread on a nutrient agar (NA) medium. Then, a paper disc (9 mm in diameter) was placed on the NA plate and pressed with 20 µL extract at the concentration of 50 mg/mL of *S. caseolaris* leaves extract. As a positive control, 10 µg Streptomycin was used on a paper disc, and Aquadest was used as a spectrum control based on the extraction solvent. The treatment was repeated three times and then incubated for 24 h at 37 °C. After that, the inhibitory power was measured by calculating the diameter of the clear zone formed [24].

Antifungal Activity

The antifungal activity of the leaves extract was carried out by the agar diffusion method by looking at the diameter of the inhibitory zone found around the perimeter of the paper disc. Tests were carried out on *Candida albicans*, and the extract concentration tested was 50 mg/mL. The culture of each test mushroom was taken from the slant using an aseptic and rejuvenated needle in a liquid medium. Each petri dish has a spore density of 105 CFU, and each culture was scraped on top of the agar. A paper disc was then placed on the agar, and 20 µL of the extract was dropped on the paper disc. The plate was incubated for 24-hours, and the obstacle zone was measured using a ruler.

Antioxidant Activity

The antioxidant activity was determined using the DPPH method according to the method used by Millauskas *et al*. [25] with some modifications. A total of 0.025 g of extract were dissolved in 10 mL of 91.67% ethyl acetate and 70% ethanol to get a concentration of 2.5 mg/mL, respectively. Then, 77 µL of the extract solution was mixed with 3 mL of DPPH (6×10^{-5} M) prepared in methanol. The mixture was then stored in a dark place for 30 min at room temperature and measured using a spectrophotometer at a wavelength of 517 nm. DPPH radical concentration was calculated using Equation 2.

$$\text{PPH radical concentration } (\%) = \frac{(A\ control - A\ sample)}{(A\ control)} \quad (2)$$

With *A control* is the value of the absorbance of the control, and *A sample* is the absorbance value of the extract tested in the sample.

Statistical Analysis

The data were subjected to the one-way analysis of variance (ANOVA), and the differences between means were determined by Duncan's multiple range test using the Statistical Analysis System (SPSS Statistics 17.0, SPSS Inc. Chicago, Illinois, USA), with $P \leq 0.05$ was regarded as significant.

3.0 RESULTS AND DISCUSSION

Phytochemical Screening

Non-nutritional vegetable chemicals with varying disease-prevention properties are called phytochemicals, valuable raw materials for traditional and orthodox medicine [26]. In this study, the phytochemical composition of the *S. caseolaris* leaves extract revealed the presence of alkaloids, saponin, tannin, flavonoid and phenolic compounds (Table 1).

Table 1 Phytochemical screening of *S. caseolaris* leaves from different solvent extracts

Phytochemical Test	Extract Solvent			
	n-hexane	Ethyl acetate	Ethanol 70%	Ethanol 96 %
Alkaloid	-	+	-	-
Saponin	-	-	+	-
Tannin	-	+	+	+
Flavonoid	+	-	+	-
Phenolic	+	+	+	+

As depicted in Table 1, alkaloid was only detected in ethyl acetate extract solvent, and saponin was detected in 70% ethanol. Tannin compound was present in ethyl acetate, 70% and 96% ethanol extract, flavonoid presence was observed in n-hexane and 70% ethanol extract, while phenolic was present in all extracts. The phytochemical test indicates that n-hexane, a non-polar solvent, can only extract flavonoid and phenolic compounds. Meanwhile, flavonoid and phenolic compounds were identified in both non-polar and semi-polar extracts, probably due to the imperfect separation in the fractionation causing the similarity of the compounds in the two extracts. The 70% ethanol extracted four compounds: saponin, tannin, flavonoid and phenolic, while 96% ethanol was only able to extract tannin and phenolic. As a universal solvent, 70% ethanol was can filter out polar and non-polar compounds.

The phytochemical compounds detected in *S. caseolaris* leaves are known to have medical properties. For example, alkaloids are reported to display anti-inflammatory [27], antimalarial [28], antimicrobial [29] and some well-known alkaloids have been included in morphine, strychnine, quinine, ephedrine, and nicotine [30]. Likewise, tannins are reported to have anti-inflammatory, bactericidal and antimicrobial activities. At present, tannins-based pharmaceutical medicine has been marketed for intestine infections due to its bactericide properties, and there are also studies of tannins as anticancer and antidiabetic [31]. Other than its antimicrobial and antioxidant properties, the potential of saponins as antiviral has been widely reported. Saponins damage the virus binding site or coating the cells, thus preventing the virus from binding to the cells. This action will directly inhibit or block the replication of the virus [32]. These properties may be the reason for using *S. caseolaris* leaves in wound healing treatment by the community of South Sulawesi, Indonesia.

Antimicrobial Activity

The agar diffusion method was used to determine the antibacterial and antifungal activity of *S. caseolaris* leaves extracts against gram-positive bacteria of *S. aureus* (Table 2), gram-negative bacteria of *E. coli* (Table 3), and a fungus of *C. albicans* (Table 4).

Table 2 The antimicrobial activity of *S. caseolaris* leaves extract against *S. aureus*

Extract concentration	Diameter Zone Inhibition from different solvent extracts (mm)			
	n-hexane	Ethyl acetate	Ethanol 70%	Ethanol 96 %
Positive control	41.7	43.22	44.54	43.54
2.5%	3.44	0.00	3.12	0.00
5.0%	3.50	0.00	6.22	0.00
7.5%	3.63	0.00	5.33	0.00
10.0%	3.73	0.00	6.98	0.00
12.5%	3.46	0.00	4.44	0.00

The antimicrobial activity was indicated by the presence of a total/partial inhibition zone around the paper disc. In Table 2, there are two extracts that have an inhibitory zone, namely n-hexane and 70% ethanol extract which showed an inhibitory effect on the growth of *S. aureus*, with the highest total inhibition being at an extract concentration of 10%. The n-hexane extract of *S. caseolaris* leaves showed the highest inhibition zone at 3.73 mm and the lowest at 2.5% concentration at 3.44 mm. Meanwhile, 70% ethanol extract of *S. caseolaris* leaves showed the highest inhibition zone of 6.98 mm. and the lowest at a concentration of 2.5% at 3.12 mm. The activity of the ethyl acetate extract *of S. caseolaris* leaves and 96% ethanol extract showed no inhibition zone, which means that the two solvents had no inhibitory effect on the growth of S. aureus. The positive control effect (Streptomycin) of each solvent had a very high inhibition zone, starting from 70% ethanol (44.54 mm), 96% ethanol (43.54 mm), ethyl acetate (43.22 mm) and n-hexane (41.7 mm).

Table 3 The antimicrobial activity of *S. caseolaris* leaves extract against *E. coli*

Extract concentration	Diameter Zone Inhibition from different solvent extracts (mm)			
	n-hexane	Ethyl acetate	Ethanol 70%	Ethanol 96 %
Positive control	29.00	28.28	33.50	29.97
2.5%	1.49	0.00	1.29	1.62
5.0%	1.04	0.00	1.04	1.25
7.5%	1.78	0.00	0.00	1.10
10.0%	1.82	1.03	1.19	1.31
12.5%	1.07	0.00	1.46	1.63
15.0%	1.07	0.00	1.57	2.18

Table 3 shows the antimicrobial activity of the *S. caseolaris* leaves extracts against *E.* coli. From the figure, n-hexane extract, 70% ethanol extract and 96% ethanol extract showed inhibition activities against *E. coli*. The 96% ethanol extract exhibited the highest inhibition effect of 2.18 mm inhibition zone against *E. coli* at a concentration of 15%. The second highest inhibition zone was 1.82 mm by 12.5% n-hexane extract. Meanwhile, a 1.57 mm inhibition zone was obtained by 70% ethanol extract at a concentration of 15%. In contrast, ethyl acetate extract does not inhibit the growth of *E. coli*.

Table 4 The antimicrobial activity of *S. caseolaris* leaves extract against *C. albicans*

Extract concentration	Diameter Zone Inhibition from different solvent extracts (mm)			
	n-hexane	Ethyl acetate	Ethanol 70%	Ethanol 96 %
Positive control	16.90	17.80	24.00	19.80
2.5%	0.00	0.00	0.00	0.00
5.0%	2.60	0.00	2.80	0.00
7.5%	2.40	0.00	4.10	0.00
10.0%	5.60	0.00	3.20	0.00
12.5%	3.10	0.00	5.00	0.00
15.0%	5.70	0.00	3.70	0.00

Table 4 shows the antimicrobial activity of different solvent extracts at different concentrations against *C. albicans*. From the table, only n-hexane extract and 70% ethanol extract of *S. caseolaris* leaves had an inhibitory effect on the growth of *C. albicans*. N-hexane extract at a concentration of 15% had the maximum inhibition zone of 5.67 mm, whereas 70% ethanol extract gave the second-highest inhibition zone of 4.95 mm. It is observed that at a concentration of 2.5%, all the solvent extract was not effective against the growth of *C. albicans*. It is also observed that all the solvents extract did not affect the growth of *C. albicans* at a concentration of 2.5%.

The results in Table 2, 3 and 4 show that there are different abilities of *S. caseolaris* leaves extract with different solvents to inhibit the growth of *S. aureus, E. coli* and *C. albicans*. This is because there is no standardisation of the manufacture of extracts of natural ingredients so that when extraction is carried out in the laboratory, the results are different. Several factors can affect the quality of extract, including chemical factors such as the type of solvent, in addition to biological factors, such as the place and origin of *S. caseolaris* leaves growth which can affect the active ingredients.

An ANOVA analysis showed that n-hexane and 70% ethanol extract of *S.caseolaris* leaves significantly ($P<0.05$) affect the growth of *E. coli*, *S. aureus* and *C. albicans*. 96% ethanol extract significantly ($P<0.05$) affected the growth of *E. coli*, while ethyl acetate extract of *S. caseolaris* leaves had no significant effect ($P>0.05$) on the growth of *E. coli*, *S. aureus* and *C. albicans*. The significant activity against the growth of *S. aureus* justified the usage of *S. caseolaris* leaves in the wound healing treatment, as *S. aureus* is known as one of the important bacteria in wound infection.

Antioxidant Activity (DPPH Scavenging Activity)

Oxidative stress is caused by the imbalance between free radical production and antioxidant defences [33], resulting in oxidative damage to biomolecules [34]. Antioxidants can reduce the oxidative stress that contributes to almost life-threatening diseases and inflammatory diseases [33]. An antioxidant is a stable molecule that donates an electron to a reactive free radical and neutralises it, thus terminating the chain reaction before any vital molecules are damaged [33] and preventing oxidative damage to target molecules [35].

This study determined the antioxidant activity of four different solvent extracts of *S. caseolaris* leaves using a DPPH radical scavenging assay. DPPH scavenging assay is based on the scavenging of DPPH. The addition of radical species or antioxidants will decolourise the DPPH solution. The potency of antioxidants is proportional to the degree of colour change. Thus, a significant decrease in spectrophotometer reading indicates a significant free radical scavenging activity [36]. Figure 1 shows the antioxidant activity for each solvent extract of *S. caseolaris* leaves. From the figure, the 96% ethanol extract showed the highest antioxidant activity of 80.21%, and ethyl acetate extract had the lowest antioxidant activity of 16.71%. Meanwhile, 70% ethanol extract had 75.89%, and n-hexane extract had 22% of antioxidant activity. The prominent antioxidant properties of the extracts are probably due to the presence of the phenolic compound in the extract [37].

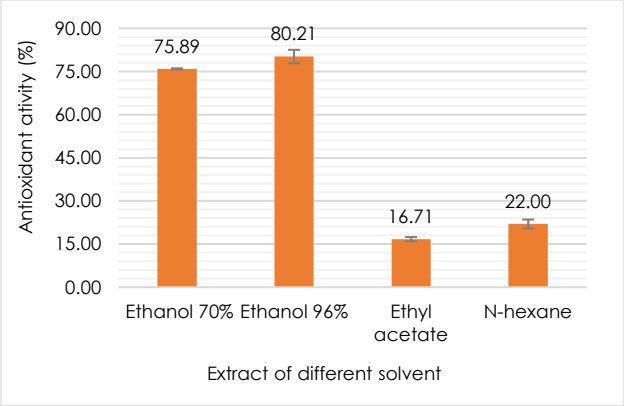


Figure 1 The antioxidant activity of different solvent extracts *S. caseolaris* leaves

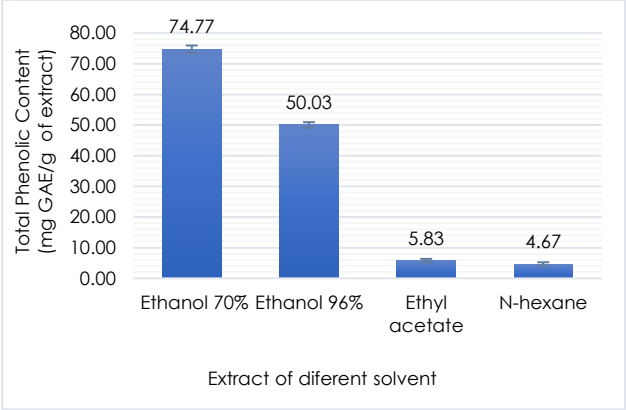


Figure 2 Total phenolic content (TPC) of the different solvent extracts of *S. caseolaris* leaves

Total Phenolic Content

The Folin-Ciocalteu (FC) method was used to determine the total phenolic content in all the solvent extracts of *S. caseolaris* leaves. The FC method determines the amount of phenolic content available in the extract based on the colour changes. The addition of Folin Ciocalteu reagent to a phenolic compound will produce molybdenum–tungsten blue measured spectrophotometrically at 760 nm. The colour intensity increases linearly with the concentration of phenolics in the reaction medium, which means high total phenolic content is present in a sample with intense blue colour [38].

Figure 2 shows the total phenolic content of different solvent extracts of S. caseolaris leaves. 70% ethanol extract has the 74.77 mg GAE/g of total phenolic content, and 96% ethanol extract has 50.03 mg GAE/g of total phenolic content. Meanwhile, ethyl acetate extract and n-hexane extract have low total phenolic content of 5.83 and 4.67 mg GAE/g, respectively. According to Labiad *et al*. [39], these results clearly show that the solvent affects the extraction ability of phenolic compounds. Plant phenolic extracts are a mixture of various phenol classes, selectively soluble in solvents. This result shows that alcohol solutions (70% ethanol and 96% ethanol) give satisfactory results for the extraction process. Ethyl acetate and n-hexane are inefficient solvents for extracting phenols from *S. caseolaris* leaves.

4.0 CONCLUSION

Five phytochemical compounds, alkaloids, tannins, saponins, phenolic and flavonoids, were successfully detected in *S. caseolaris* leaves extract. The antimicrobial test found that n-hexane extract and 70% ethanol extract of *S. caseolaris* showed significant antimicrobial activity against *E. coli*, *S. aureus* and *C. albicans*. While, ethyl acetate extract has no significant effect on the growth of *E. coli*, *S. aureus* and *C. albicans*. The highest antioxidant activity of 80.21% was detected from 96% ethanol extract, and the highest total phenolic content of 74.77 mg GAE/g was found in 70% ethanol extract. The presence of phytochemical compounds such as alkaloids, tannins and saponins, which are known for their anti-inflammatory and antimicrobial activities, and the proven antimicrobial activity against *S. aureus*, *E. coli* and *C. albicans* justified the usage of *S. caseolaris* leaves in wound treatment by the community of South Sulawesi, Indonesia.

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